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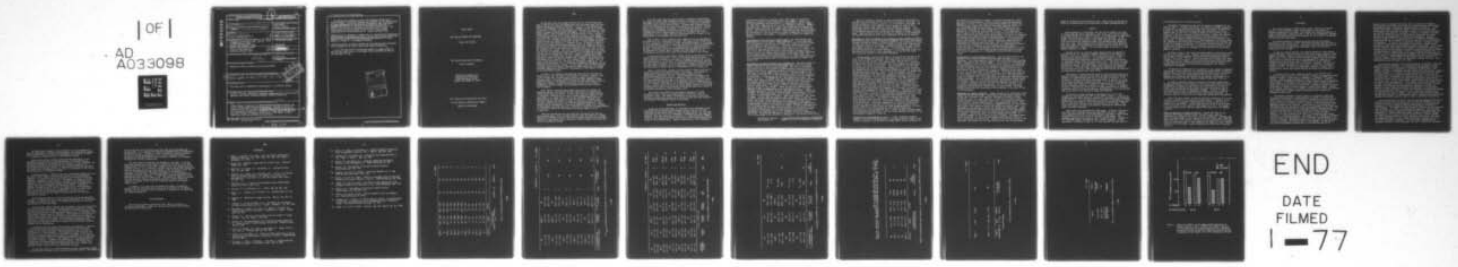

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The affects of hyperoxia at ambient pressure on thyroid function and thyroid hormone metabolism have been assessed. Thyroidal activity, as indicated by the rate of uptake of ^{125}I (I(125)) by the thyroid, was depressed in mice by exposure to hyperoxia. At 40% O_2 (+ 60% N_2) the effect was seen only after 72 hours of exposure, while at 80 - 100% oxygen, a highly significant effect was observed within 24 hours.

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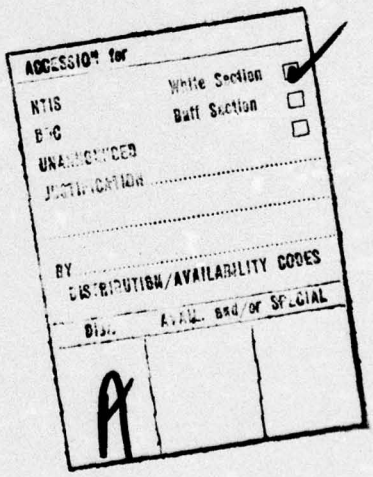
Two methods were employed to assess the peripheral metabolism of thyroid hormone. The results obtained were dependent on the concentration of oxygen employed and/or the duration of exposure. When significant changes were observed, a reduction in the rate of deiodination of thyroxine (T4) and in the deiodinative clearance of T4 occurred. The effects were seen after exposure to 40% O_2 for 96 hours; higher concentrations of O_2 were effective in shorter periods of time.

Hyperoxia also resulted in a marked fall in circulating T4 concentration and a decrease in T4-binding activity in serum. Many of these effects of hyperoxia were prevented by the concomitant administration of large amounts of vitamin E. (α-tocopheryl acetate).

These decreases in thyroid function and T4 metabolism were associated with a decrease in the rate of whole body oxygen consumption.

It was concluded that the deleterious effects of oxygen in the rat were not due, even in part, to an oxygen-induced hyperthyroid state in the peripheral tissues.



FINAL REPORT

ONR Contract N00014-73-A-0260-0002

Project NR 201-085

The Thyroid Gland and Environmental Stress in Mammals

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The discovery that high oxygen tensions can kill living tissues was made in 1878 by Paul Bert, who demonstrated that increased oxygen pressures caused convulsions and death in sparrows. For years little attention was paid to the phenomenon of oxygen toxicity. However, as the use of oxygen in medicine, in diving, and in aerospace work increased, the question of the danger of oxygen poisoning became unavoidable. At sea level pressures, 30% oxygen appears to be tolerated for an unlimited period of time. Exposure of both man and animals to 80% oxygen eventually results in mild changes in lung morphology and function, and neurological and neuromuscular symptoms can occur (1,2). However, caution must be used when the effects of increased oxygen pressure are compared in animals and man, or even between different strains of the same species (3). Brooksky, et al. (4) found no evidence of toxic effects during uninterrupted exposure of rats to oxygen at a PO_2 of 450 mm Hg, but dramatic toxic manifestations were seen at 600 and 760 mm Hg. This suggests that a rather definitive threshold exists, at least in rats, for sensitivity to oxygen. This view is supported by others (5), who found that the rat can tolerate sustained exposure to an elevated PO_2 provided the pressure does not exceed 450 - 550 mm Hg. Studies in humans also suggest that there is a tolerance level for man, probably around 450 mm Hg (6,7). Exposure to oxygen at pressures greater than 1 atmosphere can cause severe lung damage (2,7), sterility (1), and ultimately death. Indeed, death can occur in rats exposed to 60 - 80% oxygen at atmospheric pressure (3 - 6). Oxygen poisoning appears to be a time-pressure dependent phenomenon, the two conditions being inversely related. It also appears that the manifestations of oxygen toxicity are greatly influenced by other conditions; for example, associated temperature and humidity, the presence of inert gases, the age of the experimental animals.

In spite of the numerous investigations concerning the etiology of oxygen toxicity (8), the nature of the process is unclear. However, there is little doubt that increased oxygen tension produces alterations in numerous aspects of cellular metabolism and that these metabolic changes eventually lead to a disturbance of cellular function sufficiently great to produce the symptoms of oxygen poisoning seen in the intact organism. Furthermore, cellular changes can be detected before any overt signs of toxicity appear.

While details concerning the primary toxic action (or actions) of oxygen are unknown, two observations were responsible for prompting the investigations described herein. Firstly, the extent of oxygen poisoning is invariably influenced by the rate of oxygen consumption either of the whole body or of a given tissue, and can be influenced appropriately by administration of agents known to alter this rate (9). Secondly, the similarity between the effects of oxygen at high pressure and the effects of excess radiation has led to the view that hydrogen peroxide, or free radicals may be intermediates in the toxic effects of oxygen (10). Studies of peroxidation and oxygen toxicity in biological tissues and the demonstration of a protective effect of antioxidants also provide support for this concept (11).

These two observations are consistent with the possibility that the thyroid gland plays a role in oxygen toxicity, since the thyroid is of major importance in metabolic control, and recent evidence indicates that hydrogen peroxide or free radicals may be involved in thyroid hormone utilization and action in peripheral tissues.

It is well known that the thyroid gland is intimately concerned with the control of basal oxygen consumption and heat production; basal metabolic rate (BMR) varies directly with thyroid status (i.e. the rate of secretion of thyroid hormone by the thyroid gland). Although details concerning the actual site and mechanism of action of thyroxine (T_4) are unknown, from the work of this investigator and others, it is evident that there is a close, although as yet undefined, relationship between the metabolism of T_4 (deiodination) and its physiological action; an increase in the rate of deiodination of T_4 is almost invariably associated with an increase in the effectiveness of the hormone and vice-versa (12).

Other studies in this laboratory have suggested that the physiological deiodination of T_4 is mediated by an H_2O_2 - peroxidase system; deiodination of T_4 is increased in situations in which the peroxide content of the tissue is increased and the process is invariably inhibited by catalase, an enzyme which destroys tissue peroxides (12). If T_4 deiodination is indeed mediated by an H_2O_2 - peroxidase system and the metabolism and physiological action of the hormones are linked, then it follows that both processes will be influenced by conditions which alter the peroxide content of the tissues.

As indicated above, the peroxide content of the tissues may be increased under conditions of hyperoxia. Thus, an associated feature of oxygen toxicity may be an increase in the rate of T_4 metabolism and hence action. This would result in an increase in cellular oxidative metabolism, possibly into the thyrotoxic range. It is of considerable interest, therefore, that the antioxidant, vitamin E, which is beneficial in oxygen poisoning (13) has also been shown to inhibit the rate of deiodination of T_4 in vitro (14).

There is considerable evidence that the extent of oxygen poisoning is indeed influenced by the thyroid status of the animal. The toxicity is potentiated in hyperthyroidism and diminished in hypothyroid (or hypophysectomized) animals (15,16). These differences are thought to be due to the concomitant alteration in metabolic status of the animals; it has been suggested that the rate of CO_2 production and accumulation (which is increased in hyperthyroidism) is an important factor in oxygen toxicity (16,17). However, these data do not indicate whether any of the deleterious effects of oxygen are the result of an oxygen-induced increase in T_4 action and metabolism; clearly, any alteration in metabolism could be an effect of O_2 poisoning unrelated to thyroid hormone action.

The present investigation was performed to determine if oxygen poisoning is due in part to an oxygen-induced "thyrotoxic" state in peripheral tissues. The effects of hyperoxia on the rates of thyroidal secretion and peripheral metabolism of thyroid hormone have been assessed, and related to the metabolic status of the animal.

METHODS AND MATERIALS

Except when stated otherwise, all experiments were performed on male Sprague-Dawley rats weighing approximately 120 g at the start of each experiment and fed Purina Labena rat chow and water ad lib. All rats were maintained at 22°C, and in all experiments a minimum of six rats/group was employed. Rats were subjected to hyperoxia in a large hyperbaric chamber, volume 22 cubic feet which permits the control of both the pressure and the nature of the inflowing gases. Hyperoxic conditions at local atmospheric

pressure were achieved by supplying either 100% oxygen or a mixture of oxygen and nitrogen in known proportions to the chamber. Control rats for these experiments were maintained in a comparable but smaller chamber (15 cubic feet), supplied with air at local atmospheric pressure. Chambers were opened for a short period at least every second day for cleaning and feeding. After being open, the chambers were flushed with the appropriate gas at a high flow rate for 1-2 hours. Thereafter the flow rate was reduced but maintained at a rate sufficient to prevent accumulation of significant amounts of water in the chamber. A maximum of six animals per chamber was employed.

Determination of the activity of thyroid gland. Male Swiss-Webster mice (approximately 30 g) were used in these experiments. In each experiment, half were exposed to an hyperoxic atmosphere for a specific period of time, the remainder were supplied with air. Four hours before the end of the exposure period all mice received an ip injection of 1 uc ^{125}I-iodide (carrier-free NaI, New England Nuclear Corp.) given in 0.1 ml saline. Four hours later, the mice were killed by a blow and their thyroids quickly removed, placed in a counting tube and their radioactive content determined in an automatic gamma counter (Searle Analytic Inc. Model 1195). The results expressed either as cpm/thyroid/4 hr or as % dose injected/thyroid/4 hr, were analyzed statistically using methods described by Snedecor and Cochran (20).

Measurement of peripheral metabolism of exogenous T_4. Rats were brought to isotopic equilibrium with a daily subcutaneous dose of ^{125}I-T_4 of known specific activity, 2 ug/100 g body weight/day. This dose is considered approximately equal to the amount of hormone normally secreted by the rat thyroid, since it just suppresses hormonal secretion by the thyroid and is sufficient to achieve normal plasma T_4 levels. Thyroidal uptake of any ^{125}I released from the ^{125}I-T_4 was prevented by giving 1% $KClO_4$ in place of drinking water. ($KClO_4$ *per se* does not significantly alter the peripheral metabolism of T_4). The metabolism of T_4 was assessed as follows. The animals were placed in special individual metabolism cages which permitted the separate collection of urine and feces. Serial 24-hr collections of urine were made and a sample of tail blood was obtained at the end of each collection period. The amount of ^{125}I in urine was determined by counting aliquots in the counter. Iodide was the only labelled compound detected, either chromatographically or electrophoretically, in urine. The amount of organic ^{125}I in serum was determined as follows: organic and inorganic ^{125}I were separated by short-term paper electrophoresis (90 min) in 0.05 M glycine-acetate buffer, pH 8.6 using a constant current of 12 milliamps. NaI was added as a marker. Under these conditions, the iodide, located by staining with palladium chloride, moved several cm from the origin; the organic iodine remained at the origin. The appropriate sections of the strips were cut and the radioactivity determined in the counter. Chromatographic analysis of the serum in a butanol-dioxane-2N NH_4OH system (4:1:5) revealed that at least 99% of the organic ^{125}I was in the form of T_4. From these values and the known specific activity of the injected ^{125}I-T_4, the absolute concentration of T_4 in the serum and the quantity of hormone deiodinated (as estimated from the ^{125}I excreted in the urine) were calculated. The data were then used to calculate the rate of clearance of ^{125}I-T_4 from plasma by the deiodinative pathway [i.e. as iodide in urine (21)]:

$$\text{deiodinative clearance (ml plasma/24 hr)} = \frac{\text{urinary } ^{125}\text{I (\% } ^{125}\text{I injected as } T_4\text{/day)/24 hr}}{\text{plasma } ^{125}\text{I-T4 (\% } ^{125}\text{I injected as } T_4\text{/day)/ml}}$$

With this technique, specific activity equilibrium (as indicated by a constant rate of excretion of ^{125}I in urine) is achieved in 10-14 days. At this point hyperoxic stress was applied. In a few initial experiments, the animals were placed in the chambers in their metabolism cages so that collections could be made during the period of hyperoxic exposure. This proved impractical as only four cages could be inserted together. Thus in the experiments reported here, the animals were exposed to oxygen in regular cages. They continued to receive the daily T_4 injection, and T_4 metabolism was determined during the 24-hr period immediately following the exposure.

Measurement of rate of turnover of T_4 following a single injection of ^{125}I-T_4. The technique using rats equlibrated with exogenous ^{125}I-T_4 has limitations; although the absolute amount of hormone metabolized can be measured precisely, the daily dose has to be selected, it is given a single daily injection, and no allowance is made for any change in thyroid secretion rate under altered experimental conditions (i.e. hyperoxia). Thus peripheral turnover of T_4 was also determined by the second technique. In this technique, widely used for assessing T_4 turnover in man, endogenous T_4 is labelled with tracer ^{125}I-T_4 and the kinetics of T_4 turnover determined.

The measurement requires three days so this period was included in the total period of hyperoxic exposure. Rats were given an IV injection of a tracer dose of ^{125}I-T_4 (less than 0.01% of total body content of T_4) at 4.0 pm on the first of the 3 days, together with 1 mg NaI in 0.1 ml saline, to prevent ^{125}I uptake by the thyroid. Tail blood samples were obtained at 8.0 am and 8 pm on the next two days. (This allowed 16 hr for the tracer to equilibrate with the extrathyroidal endogenous T_4). The NaI injection was repeated on each of these days and the animals continued to receive the appropriate gaseous mixtures. After the last blood sample, the animals were exsanguinated under ether anesthesia. Serum was obtained and the T_4 content determined by the local radioimmunoassay laboratory (courtesy of Dr. Truls Brink-Johnson). Liver, kidney and muscle tissue were taken for the studies of deiodination in vitro described below. The ^{125}I-T_4 content of sera from the tail blood samples was determined by the electrophoretic technique described above and the percentage of the injected ^{125}I-T_4 in each sample was calculated. For each rat, the rate of disappearance of ^{125}I-T_4 from the serum was plotted on semi-log paper. After the injected ^{125}I-T_4 is equilibrated with the endogenous T_4, a straight line can be obtained using the method of least squares analysis. The theoretical plasma concentration of ^{125}I-T_4 at 0 time is determined by extrapolating this line to 0. The slope of the line is the fractional disappearance rate of T_4 (k), the reciprocal of the 0 time extrapolate (100/% dose/ml) is the total distribution space (TDS). The metabolic clearance rate (MC)=k x TDS. These turnover studies were carried out in the metabolism cages and both urine and feces were collected. The urinary radioactivity was determined as described above. Feces were homogenized in a mixture of human plasma (to bind any free T_4) and water (1:4) to yield a total volume of 60 ml. Protein bound ^{125}I in feces (which was shown chromatographically to consist entirely of ^{125}I-T_4) was determined by the standard technique of trichloroacetic acid precipitation (18). Urinary and fecal clearance rates were calculated using the mean of the four values obtained for serum ^{125}I-T_4 concentration (%dose/ml) in each rat.

Assessment of T_4 deiodination in vitro: In order to determine whether changes in the rate of deiodination of T_4 in vivo reflected a change in the intrinsic activity of the deiodinating enzyme rather than an alteration in

the intensity of T_4 binding to protein in serum and extravascular fluids, the rate of T_4 deiodination by isolated tissues in vitro was determined. Homogenates of liver, kidney and muscle from the control and treated rats from the in vivo studies were made in Krebs-Ringer phosphate buffer, pH 7.0, containing 2 mg/ml glucose (KRPG). T_4-deiodinating activity was measured by incubating the tissue homogenates (1 ml, 1:100 wt/vol) with radioactive T_4 (10^{-9}M) for known periods of time. ^{125}I-T_4 (Amersham-Searle) comes in 50% propylene glycol and appropriate dilutions in 0.01% human serum albumin were made for in vitro studies. At the end of incubation the reaction was stopped by addition of human plasma (0.3 ml), which binds any available hormone and prevents further metabolism. The percentage of organic and inorganic ^{125}I in the reaction mixture was determined by the electrophoretic technique described above. The ^{125}I-labelled products formed were also determined in some experiments by chromatographic analysis. T_4 and inorganic iodide were the only labelled compounds present in significant amounts. The percentage of the ^{125}I-T_4 deiodinated by a tissue preparation was corrected for iodide generated non-enzymically in tissue-free control incubations, or in incubations carried out with pre-boiled tissue.

Determination of serum T_4-binding activity. Serum T_4-binding activity was assessed using a charcoal-binding assay technique developed in this laboratory. Each serum sample was diluted 1:500 with Krebs-Ringer phosphate buffer containing 2 mg/ml glucose (KRPG). 1 ml aliquots were incubated with ^{125}I-T_4 (added in 10 ul to yield 10^{-8}M) for 30 min at 37°C. The percentage of labelled hormone bound to tissue components was determined by mixing an aliquot of labelled serum (100 ul) with 100 ul charcoal solution (1.0 g Norit Neutral and 0.1 g Dextran 60 in 100 ml 1.5 mM $MgCl_2$) prechilled to 4°C in a 1.5 ml capped plastic tube (Eppendorf). The mixture was shaken on a vortex agitator for 5 sec, held at 4°C for 60 min and then centrifuged at 4°C in an Eppendorf centrifuge (Model 3200) for 2 min. The proportion of organic to inorganic ^{125}I in the supernatant was determined electrophoretically as described above. The total counts and their distribution between organic and inorganic iodide in the serum mixture were determined by revortexing a 1:1 mixture of charcoal solution and labelled serum after holding at 4°C for 60 min, electrophoresing and counting. The ^{125}I-T_4 remaining in the supernatant represented that tightly bound to tissue protein. Relative binding activity was thus caluclated as the percentage of the total ^{125}I-T_4 in the supernatant.

Measurement of oxygen consumption. Oxygen consumption was determined in rats exposed to 60% and 100% oxygen. Measurements were made before exposure and on each day of exposure in an apparatus purchased from Medical Science Electronics, Inc., St. Louis, Missouri. It consists of a small plastic chamber attached to a volume meter (Model 160). The chamber was maintained at a relatively constant temperature by a surrounding water jacket. Soda lime (Fisher Scientific Co.) and Drierite (Hammond Drierite Co., Xenia, Ohio) were placed in the chamber to absorb the exhaled CO_2 and water vapor. The measurements were made after flushing the chamber with 100% oxygen. Oxygen uptake is recorded on graph paper by the pen mechanism of the volume meter; the pen moves horizontally at a rate reflecting the rate of oxygen uptake in the chamber. The pen also makes a vertical movement every minute. A horizontal movement of 1cm is equal to 0.673 ml oxygen. Thus oxygen uptake can be calculated. For each rat, measurements were made for 30-60 min, thus allowing the rat to setle down. Because of the stressful nature of the experiment, the animals were not food-restricted and thus the measurement

cannot be considered a basal metabolic rate. Values given are the mean of 30-60 min of observations and are expressed as oxygen consumed/minute/rat.

RESULTS

During exposure to O_2 (40-100%), the rats did not appear to be under an obvious physical strain comparable to that which occurs during exposure to altitude (18,000 ft) or cold 4°C (23,24). At 40-60% oxygen no significant changes in food and water intake or body weight were observed. At 80% oxygen, there was some decrease in food intake and body weight during the first 24-hr period but thereafter normal food consumption was resumed at least for 5 days, (the maximum period of exposure to 80% oxygen employed). With 100% oxygen, food consumption was reduced during the first 24 hr, but improved thereafter. However, 150 g rats were unable to survive in a 100% oxygen atmoshpere for 5 days.

Exposure of mice to increased oxygen concentrations at atmospheric pressure resulted in a decrease in thyroid function, as indicated by a decrease in the rate of uptake of ^{125}I by the thyroid gland (Table 1). Thyroid uptake was measured over a 4-hr period to minimize the error introduced when newly synthesized labelled ^{125}I-T_4 begins to be secreted in significant amounts from the gland (after 8 hrs). The effect of oxygen was dependent on both the concentration of the gas in the mixture and the duration of exposure. With 100% oxygen, thyroid function was depressed within 24 hrs. With 80% oxygen, a significant depression was generally not observed until 48 hrs. When 40% oxygen was administered, an effect was not consistently observed until after 72 hrs.

The decrease in thyroid function which occurred following exposure to hyperoxia could be prevented to some extent by administration of Vitamin E. Vitamin E was administered either as a sc injection of Vitamin E in oil (20 mg/rat); controls received an equivalent amount of vehicle, or in the food (20 mg α-toxopheryl sulphate/10 g food). In a series of experiments, it was found that Vitamin E was ineffective when given only during the period of hyperoxic exposure. However, as shown in Figure 1, if Vitamin E was given in the diet for 3 days prior to oxygen exposure and in addition, injected on each of the days of exposure, the depression of thyroid function seen in the control mice exposed to oxygen was prevented.

The effects of oxygen exposure on the metabolism of T_4 in rats isotopically equilibrated with ^{125}I-T_4 are shown in Table II. Exposure to 40-80% oxygen for 96 hrs generally resulted in a decrease in the peripheral metabolism of T_4. This was manifested in a decrease in the amount of iodide derived from T_4 excreted in urine and usually this decrease was associated with a significant increase in the concentration of T_4 in serum. These values were used to calculate the urinary or deiodinative clearance was almost invariably decreased.

Some studies were performed with 100% oxygen. In these, there was an indication that T_4 metabolism was decreased within 36-48 hrs. However, these experiments were not satisfactory because a decrease in food intake occurred during the first 24 hrs of exposure to 100% oxygen. This phenomenon *per se* has been shown to alter T_4 metabolism (22). Attempts to pair-feed were largely unsuccessful due to animal variation. In the experiments illustrated in Table II, all rats consumed the same quantity

of food during the 24 hr collection period.

The effects of hyperoxia on the rate of turnover of endogenous T_4 are shown in Table 3. In these experiments, rats exposed to 80-100% oxygen were at least 250 g; they withstood the oxygen stress much better and were more suitable for the repetitive bleedings than were the small rats (150 g). Hyperoxia for 3-7 days had no consistent effect on the fractional rate of turnover of ^{125}I-T_4 or the total distribution space. However, the exposure invariably resulted in a marked decrease in the concentration of T_4 in serum. Thus the absolute amount of hormone utilized in the hyperoxic rats was reduced. It should be noted that in these experiments the thyroid gland was functioning and the decrease in serum T_4 (unlabelled) was presumably due in part to a decrease in the rate of T_4 secretion from the thyroid. In the animals equilibrated with ^{125}I-T_4, no provision was made to alter the daily supply of T_4 to simulate this decrease, and thus the serum T_4 levels were generally elevated.

As shown in Table 3, the calculated total metabolic clearance was rarely altered in the hyperoxic rats. However, changes were generally observed in the urinary and fecal clearances calculated from the measurements made of urinary ^{125}I and fecal ^{125}I-T_4. Urinary clearance was decreased while fecal clearance of T_4 was increased. Again, since the serum T_4 levels were reduced in the hyperoxic rats, these data indicate that the absolute amount of T_4 removed from the plasma by the deiodinative pathway per unit time was significantly decreased.

The decrease in deiodination of T_4 observed in rats exposed to hyperoxia could, under certain conditions, be prevented by administration of Vitamin E. Administration of Vitamin E only during hyperoxic exposure was insufficient. However, if Vitamin E was administered in the food for 3 days prior to exposure and in addition was injected during exposure, the decrease in T_4 metabolism resulting from the hyperoxia became insignificant (Table 4).

In order to determine whether the decrease in hormonal deiodination in vivo reflected an actual decrease in the intrinsic deiodinating activity of peripheral tissues or was due merely to an alteration in the rate of transport of the hormone to the tissues, the T_4-deiodinating activity of liver, kidney and muscle was determined in vitro. As shown in Table 5, the deiodinating activity in homogenates of muscle (1:100 w/v) prepared from rats exposed to 80% O_2 for 48 hrs was significantly decreased. In contrast, activity in both liver and kidney were unaffected by the exposure.

One of the major factors influencing peripheral metabolism of T_4 is the intensity of binding of the hormone to serum proteins. A change in serum binding activity is generally associated with a change in the rate of metabolism of the hormone. A significant decrease in the serum T_4-binding activity occurred in rats exposed to 80% oxygen for 48 hrs. The changes in serum binding activity were not apparent after only 24 hrs of exposure (Table 6).

Effect of hyperoxia on oxygen consumption. Studies were made on rats exposed to 60% oxygen and 100% oxygen. With 60% oxygen exposure, measurements were made on each of the five days of exposure. Although, the means of values obtained in the exposed rats on days 4 and 5 were invariably lower than that of the controls, the data were not statistically significant. Similar findings were made in rats exposed to 80% oxygen for 1 day. However, after 48 hrs in 80% oxygen, oxygen consumption was significantly depressed (Table 7).

DISCUSSION

The results obtained in these studies clearly indicate that several aspects of thyroid hormone economy are decreased in rats exposed to elevated concentrations of oxygen. The effects were induced by exposure to oxygen concentrations ranging from 40% to 100% at local atmospheric pressure. However, considerably more time was necessary for the effects to be manifest with 40% than with 100% oxygen.

The methods employed for assessing thyroid function and hormonal metabolism in these studies are well established and many have been used in this laboratory for several years. Thus most of the associated problems and pitfalls are known and appropriate adjustments either in technique or interpretation can be made.

To test for thyroid function, the standard radioactive iodine uptake test was employed. Mice were chosen for this study because relatively large numbers are needed. There is also less variation in iodine uptake in mice than in rats. In the hyperoxic mice thyroidal uptake of ^{125}I was decreased. The possibility that this was due not to depressed ^{125}I uptake but to an enhanced rate of secretion of newly synthesized ^{125}I-T_4 was virtually excluded by measuring uptake over a 4 hr period. Normally, ^{125}I-T_4 is not present in significant amounts in plasma until at least 8 hrs following injection of ^{125}I. In two of the experiments, absence of organic ^{125}I in plasma of both control and exposed mice was confirmed by electrophoretic analysis.

A second pitfall in this technique relates to the $^{125}I/^{127}I$ ratio achieved following injection of the isotope. Provided the endogenous iodide content of animals in each group is comparable, a change in ^{125}I uptake reflects a similar change in uptake of stable iodine (^{127}I). However, if the treatment or experimental conditions induce a marked change in endogenous iodide content, it is possible for the specific activity achieved to be sufficiently different from the controls that a change in radioactive iodide uptake does not reflect a similar change in stable iodide uptake. However, in the present experiments, any changes in food and water intake and excretion were insufficient to warrant concern regarding serious alterations in endogenous iodide levels.

The apparent decrease in thyroid function in hyperoxic animals was invariably associated with a marked decrease in plasma T_4 concentration. Theoretically a decrease in circulating T_4 concentration can result either from a reduction in the rate of secretion of T_4 by the thyroid or from a decrease in the T_4-binding activity in plasma. In these experiments both phenomena appeared to be involved. Thyroid function as indicated by the ^{125}I-uptake test, was decreased, and by direct measurement it was found that the total T_4-binding activity in plasma was reduced in exposed animals. It is well known that most of the T_4 circulating in plasma is bound to serum proteins; less than 1% of the total plasma T_4 is in the free form. However, it is the concentration of free T_4 in plasma that under normal conditions is maintained constant. For example, if an alteration in binding activity occurs, the percentage of free T_4 and hence the concentration of free T_4 is charged. Thyroidal secretion of T_4 must then be altered acutely to restore to normal the concentration of free hormone. At this point a new equilibrium between bound and free T_4 is established and the T_4

secretion rate can resume its original level. In this way a change in binding activity and hence total plasma T_4 concentration can occur without any permanent change in thyroidal secretion rate or T_4 turnover. In the present experiments, although the change in binding activity must have contributed to the reduction in plasma T_4 levels, it is unlikely that this was entirely responsible for the change. Firstly, the decrease in binding activity was relatively small and was probably insufficient to account entirely for the observed drop in plasma T_4 concentration. Secondly, thyroidal uptake of ^{125}I was decreased and it is therefore reasonable to assume that the rate of release of T_4 by the gland into the circulation was also decreased. It should also be noted that the fall in plasma T_4 levels was masked to some extent by the decrease in peripheral turnover of the hormone which must have counteracted the change to some extent. Some insight into the relative contribution of the changes in T_4 secretion rate and T_4 binding activity to the decrease in plasma T_4 concentration could be obtained by determining the concentration of free T_4 in plasma of exposed rats. A decrease in free T_4 concentration would suggest that the pituitary-thyroid axis is unable to respond adequately and restore the free T_4 concentration to its normal value. Unfortunately determination of free T_4 levels in rat plasma is difficult since there is a great deal of animal variation. Such determinations were not made in this study.

The peripheral metabolism of T_4 was assessed by two methods. Both have advantages and disadvantages. The isotopic equilibrium method is useful because it enables the investigator to determine the metabolism of known amount of hormone in the absence of a functioning thyroid gland. Thus unknown variations in endogenous hormone secretion rate are avoided. However, with this method, it is necessary to make a decision concerning the amount of hormone required to simulate the physiological level, a task that is not always easy. Furthermore, the continuous secretion of T_4 by the thyroid cannot be mimicked precisely when hormone is supplied by injection once (or twice) a day. In most cases, however, these problems are not serious and can even be used advantageously. In the present study it was evident that the dose of T_4 administered was on the high side since the serum T_4 levels achieved in the exposed rats were generally increased; in normal rats exposed to oxygen, plasma concentration of endogenous T_4 was decreased. Since it has been shown that in normal rats the amount of hormone deiodinated is proportional both to the dose of T_4 and to the serum concentration of T_4, when the dose range is between 1 and 6 ug/100 g body weight/day (25), the observed decrease in T_4 deiodination and its clearance from plasma in the presence of elevated plasma T_4 levels in rats is particularly convincing.

The second method utilized the intact rat and was employed primarily to confirm and substantiate the results of the study with exogenous T_4. The technique provides information regarding many aspects of thyroid hormone economy and the data obtained emphasizes the importance of detailed investigations. For example the total metabolic clearance and the fractional rate of turnover of T_4 were not influenced by hyperoxia. On the surface these findings appear to contradict those obtained in the experiments with endogenous T_4. However, these measurements reflect the combined turnover or clearance of T_4 by both the urinary (deiodinative) and fecal pathway. The latter represents merely a loss of hormone to the system. When each was determined directly it was evident that urinary clearance was, as in the other experiments, depressed. Since there was a concomitant and comparable increase in fecal clearance, the total clearance was unchanged.

The significant increase in fecal clearance of T_4 was unexpected in view of the decrease in plasma T_4 concentration. This observation suggests that the handling of T_4 by the liver (conjugation and excretion in bile) is in some way influenced directly in the hyperoxic animals.

These tracer turnover studies substantiate the observation in the equilibration studies that the absolute amount of T_4 deiodination was decreased; although the fractional rate of turnover of T_4 was unchanged, the plasma T_4 concentration was decreased, thus the actual amount of T_4 deiodinated must have been reduced. It was therefore concluded from these two studies that the hyperoxia in rats results in a decrease in the rate of deiodination of T_4 in peripheral tissues.

As mentioned above, a change in the rate of peripheral metabolism of T_4 can result from a change in the intrinsic activity of the deiodinating mechanism. It also can be due to an alteration in the rate at which the hormone is transported in the tissues. For example, if plasma T_4 binding activity is decreased, there follows an increase in the percentage and temporarily also in the absolute amount of free hormone in plasma and this rapidly equilibrates throughout the peripheral tissues. Conversely a decrease in binding activity results in a reduction in the percentage of free T_4 in plasma and hence also in peripheral tissues. In the present work, the decrease in peripheral deiodination of T_4 could not be attributed to an increase in plasma T_4-binding activity since binding activity was reduced.

The deiodinating activity of several individual tissues was measured in vitro. It appears that the tissue primarily responsible for the decrease in rate of deiodinating is muscle. No significant alteration in activity was observed in liver and kidney.

These findings clearly indicate that the hyperoxic rat is not thyrotoxic; both the action and metabolism of T_4 were depressed in peripheral tissues and thyroid activity was reduced. The decrease in thyroidal activity could not be attributed solely to the decreased turnover of T_4 in peripheral tissue since serum T_4 levels were also decreased. The reduction in circulating T_4 concentration suggests that the pituitary-thyroid system, which normally serves to maintain serum T_4 levels, was not operating adequately under these conditions. However, the decrease in thyroid function could also have resulted from a decrease in the rate of secretion of thyrotropin-releasing hormone from the hypothalamus.

The data also do not support the view that T_4 deiodination is mediated by an H_2O_2-peroxidase system since under conditions in which the H_2O_2 content of the tissues has been found to be elevated (10), the rate of deiodination of T_4 was decreased. The decrease, at least in muscle, was intrinsic to the tissue and was not the result of changes in the rate of delivery of hormone to the tissue (indeed in some studies it was found that the concentration of ^{125}I-T_4 in liver and muscle was the same in hyperoxic and control animals). Therefore, the potential for T_4 deiodination to be increased by peroxidase mediated system was present. The finding that deiodination was actually decreased under these conditions suggests that H_2O_2 has little, if any, role in T_4 deiodination in vivo.

On the other hand, as is almost invariably the case, concomitant changes in T_4 action and T_4 metabolism were observed; both were reduced. Unfortunately,

one can conclude little from this other than that the two phenomena may be associated. It is possible that hyperoxia interferes with T_4 deiodination and hence its action. Conversely, since T_4 influences oxidative processes in the cell and the deiodination of T_4 is also an oxidative process, hyperoxia may interfere primarily with T_4 action, resulting in a secondary alteration in the rate of metabolism.

How these effects of hyperoxia are mediated is not clear. It is well known that exposure of man and animals to hyperoxic conditions of the type employed herein results in only small changes in pO_2 in all tissues with the exception of lung. It is difficult to accept that the deiodinative metabolism would be decreased directly by small changes in pO_2, particularly since the activity of the mechanism, when studied *in vitro* is increased in an atmosphere of 95% O_2 (26). Evidently the effects of O_2 on deiodination *in vivo* and *in vitro* are different. It seems more likely the depression of T_4 deiodination is an indirect effect of O_2. The possibility cannot be exculded that the peripheral effects of hyperoxia observed in these studies are secondary to changes in the lung, the tissue that exhibits the greatest change in pO_2.

In summary, it is clear that the deleterious effects of hyperoxia *in vivo* cannot be attributed, even in part, to an increase in the thyroid function. On the contrary, one of the effects of hyperoxia is to render the rat somewhat hypothyroid.

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TABLE I

Effect of hyperoxia on thyroid function in mice

Expt.	% oxygen	Hr. exposed	Thyroidal ^{125}I - uptake (cpm/gland/4hr)		
			Control	Exptl.	p value
1	100	24	327 ± 37+	238 ± 22	NS
2	100	48	327 ± 37	216 ± 11	< 0.025
3	100	24	252 ± 26	142 ± 14	< 0.001
4	80	24	85 ± 10	61 ± 5	NS
5	80	48	85 ± 10	50 ± 5	< 0.05
6	80	24	1145 ± 65	901 ± 93	< 0.025
7	60	48	1054 ± 189	869 ± 87	NS
6	60	48	1566 ± 62	1060 ± 64	< 0.001
7	60	72	1566 ± 62	1024 ± 72	< 0.001
8	60	48	1451 ± 65	824 ± 75	< 0.001
9	50	48	4170 ± 467	701 ± 239	< 0.001
10	40	72	1481 ± 598	753 ± 50	NS
11	40	72	3631 ± 252	2351 ± 224	< 0.005

+ Mean ± se

TABLE 2

Effect of hyperoxia on T_4 metabolism in rats isotopically equilibrated with ^{125}I-T_4

Expt.	% oxygen	Duration of exposure(hr)	Group	Urinary I^- (μg ≡ T_4/24 hr)	Serum T_4 (μg/100ml)	Urinary clearance of T_4 (ml plasma/24 hr)
1	80	96	C	1.4 ± 0.075[+]	1.9 ± 0.015	75.9 ± 7.9
			E	0.9 ± 0.087	2.0 ± 0.021	45.9 ± 7.9
				< 0.001	NS	p < 0.025
2	80	96	C	1.4 ± 0.06	2.0 ± 0.6	76.0 ± 4.9
			E	1.1 ± 0.05	3.0 ± 0.2	46.5 ± 5.6
				< 0.005	< 0.01	< 0.001
3	40	96	C	1.5 ± 0.05	3.0 ± 0.4	62.2 ± 3.1
			E	1.3 ± 0.08	3.0 ± 0.5	49.6 ± 2.1
				< 0.05	NS	< 0.05
4	40	96	C	0.98 ± 0.02	2.7 ± 0.1	34.8 ± 1.6
			E	0.92 ± 0.03	3.5 ± 0.2	26.4 ± 2.3
				NS	< 0.005	< 0.01
5	40	110	C	1.63 ± 0.09	5.5 ± 0.3	25.9 ± 1.7
			E	1.42 ± 0.32	6.4 ± 0.2	24.1 ± 1.8
				NS	< 0.025	NS

+ Mean ± se C - controls; E - O_2-exposed

TABLE 3

Thyroxine turnover studies in rats exposed to hyperoxia

	Expt	Group	k	TDS (ml)	Metabolic Clearance	Urinary Clearance	Fecal Clearance	Serum T_4 Concentration µg/100 ml
					(ml plasma / 24 hr)			
I	100% O_2	C	0.44 ± 0.037[+]	15.3 ± 0.73	6.71 ± 0.49	3.9 ± 0.30	2.76 ± 0.26	4.7 ± 0.48
	3 days	E	0.43 ± 0.03	16.6 ± 1.32	7.05 ± 0.42	3.2 ± 0.38	3.90 ± 0.24	3.4 ± 0.32
			NS	NS	NS	NS	< 0.01	< 0.05
II	100% O_2	C	0.40 ± 0.03	13.2 ± 0.79	5.21 ± 0.22	3.24 ± 0.19	1.96 ± 0.18	4.9 ± 0.33
	3 days	E	0.54 ± 0.02	9.38 ± 0.29	5.04 ± 0.11	2.61 ± 0.13	2.43 ± 0.09	2.8 ± 0.20
			< 0.005	< 0.005	NS	< 0.025	< 0.05	< 0.001
III	80% O_2	C	0.48 ± 0.02	18.3 ± 1.28	8.74 ± 0.53	4.99 ± 0.23	3.75 ± 0.36	5.7 ± 0.32
	4 days	E	0.45 ± 0.01	19.7 ± 1.14	8.99 ± 0.55	5.77 ± 0.32	3.19 ± 0.24	4.3 ± 0.27
			NS	NS	NS	NS	NS	< 0.01
IV	80% O_2	C	0.45 ± 0.006	13.7 ± 0.39	6.16 ± 0.13	3.8 ± 0.13	2.36 ± 0.13	3.95 ± 0.27
	5.5 days	E	0.48 ± 0.026	12.3 ± 0.42	5.92 ± 0.24	3.5 ± 0.35	2.24 ± 0.19	2.65 ± 0.24
			NS	< 0.05	NS	NS	NS	< 0.005
V	80% O_2	C	0.45 ± 0.016	12.62 ± 0.79	5.66 ± 0.20	4.61 ± .36	1.62 ± 0.13	3.28 ± 0.29
	7 days	E	0.42 ± 0.023	12.13 ± 0.34	5.06 ± 0.20	3.26 ± .36	2.79 ± 0.13	3.15 ± 0.24
			NS	NS	< 0.05	< 0.025	< 0.01	NS

[+] Mean ± se C - controls; E - O_2-exposed

TABLE 4

Effect of Vitamin E on T_4 metabolism in rats exposed to hyperoxia

Expt	O_2 exposure (hr)	Group	Urinary I^- (μg ≡ T_4)	Serum T_4 (μg/100 ml)	Urinary clearance of T_4 (ml plasma/24 hr)
1	24	Cont	0.65 ± 0.04+	1.6 ± 0.09	93.0 ± 12.8
		Cont + O_2	0.46 ± 0.04	1.7 ± 0.07	90.0 ± 11.6
			NS	NS	NS
		Vit E	0.63 ± 0.021	1.7 ± 0.05	80.5 ± 6.5
		Vit E + O_2	0.70 ± 0.11	1.8 ± 0.07	95.3 ± 20.1
			NS	NS	NS
2	48	Cont.	0.87 ± 0.058	0.7 ± 0.04	109.7 ± 7.85
		Cont + O_2	0.61 ± 0.035	0.8 ± 0.04	68.1 ± 4.8
			<.0.001	NS	< 0.001
		Vit E	0.848 ± 0.11	0.8 ± 0.037	90.6 ± 14.3
		Vit E + O_2	0.841 ± 0.05	1.2 ± 0.18	79.7 ± 13.0
			NS	NS	NS

+ Mean ±se

TABLE 5

Effect of hyperoxia *in vivo* on deiodinating activity in isolated tissue preparations *in vitro*

Extp.	Duration of exposure (hr)	Tissue	Deiodinating activity (% T_4 deiodinated)		
			Control	Exptl.	p value
1	48	Muscle	25.2 ± 2.20[+]	18.4 ± 0.66	< 0.025
	48	Liver	04.2 ± 3.20	67.1 ± 4.76	NS
	48	Kidney	32.2 ± 3.1	30.7 ± 4.2	NS
2	48	Muscle	30.5 ± 3.18	18.6 ± 1.8	< 0.01
	48	Liver	63.3 ± 9.54	68.2 ± 1.53	NS

+ Mean ± se. Deiodinating activity was determined in homogenates, 1:100 wt/vol prepared in KRPG pH 7.0. ^{125}I-T_4, 10^{-9} M was employed. Incubations were carried out for 1 and 2 hr. (2 hr value shown here) under 95% O_2: 5% CO_2.

Table 6

Effect of hyperoxia or serum T_4-binding activity

Expt.	Duration of Exposure (hr)	Binding activity (^{125}I-T_4 in supernatant) (% total control)	Expt.	P value
1	48	56.6 ± 1.67[+]	46.8 ± 1.6	< 0.001
2	48	40.9 ±	32.5	< 0.005

+ Mean ± se

TABLE 7

Effect of hyperoxia on oxygen consumption in rats

Treatment	Oxygen uptake ml O_2/min/rat
Air	5.43 ± 0.24
Oxygen (100% 48 hr)	3.98 ± 0.15
p value	< 0.001

Mean ± S.E.

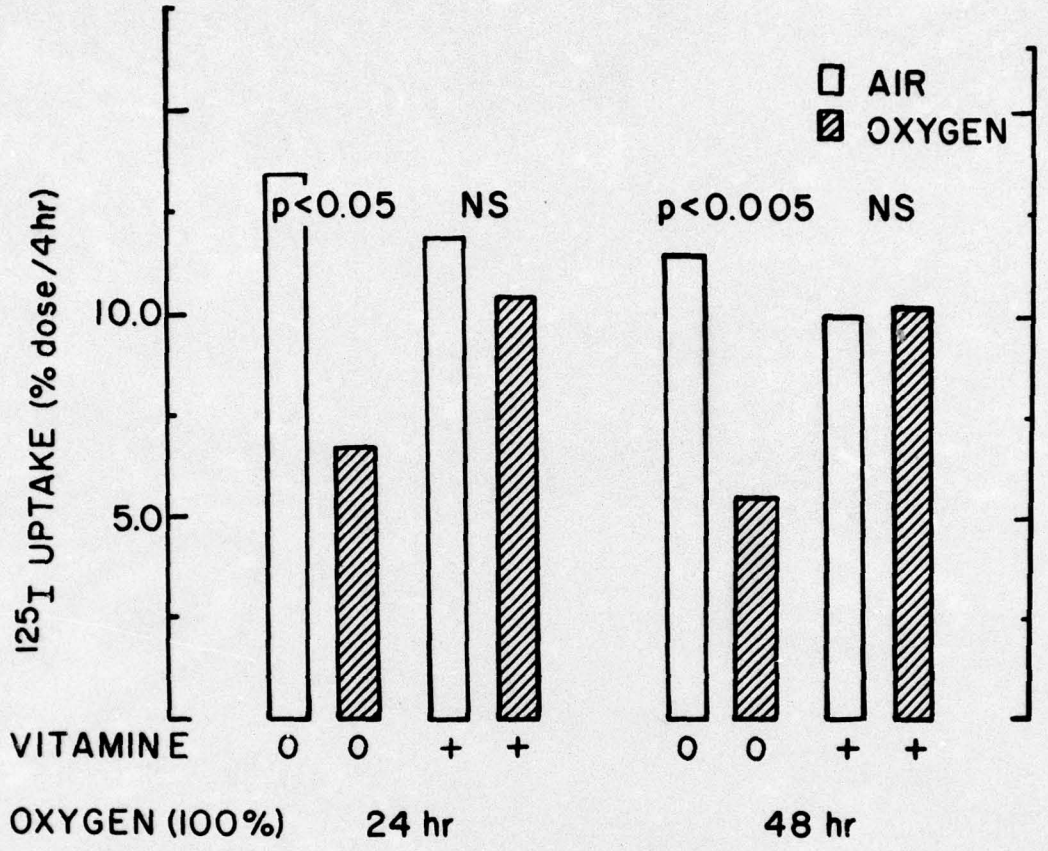

Figure 1. Effect of vitamin E on the oxygen-induced depression of thyroid function in mice. Vitamin E was given in the diet for three days prior to oxygen exposure (20 mg α-tocopheryl sulphate/10 g food) and was also injected on each of the days of exposure (20 mg Vitamin E in 0.25 ml sesame seed oil/rat).